

Applicant: SCARINGE, Stephen  
Serial No.: 10/635,108  
Filing Date: August 5, 2003  
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**Amendments to the Claims:**

This listing of claims will replace all prior versions and listings of claims in the application:

**Listing of Claims:**

1.-8. (canceled)

9. (currently amended) A method for inhibiting an mRNA, comprising:

a) providing an RNA comprising the structure  $X_1$ -L- $X_2$ , wherein  $X_1$  and  $X_2$  are nucleotide sequences having sufficient complementarity to one another to form a double-stranded stem hybrid and L is a flexible loop region comprising a non-nucleotide linker molecule of 10-24 atoms in length, wherein at least a portion of one of the nucleotide sequences located within the double-stranded stem is complementary to a sequence of the target mRNA; and

b) contacting the RNA comprising the structure  $X_1$ -L- $X_2$  with a sample *in vitro* containing or suspected of containing the target mRNA under conditions that favor transfection of the RNA comprising the structure  $X_1$ -L- $X_2$  into a cell comprising the target mRNA whereby presence of the RNA comprising the structure  $X_1$ -L- $X_2$  decreases expression of the target mRNA;

wherein  $X_1$  and  $X_2$  each independently comprise between about 19 to 27 nucleotides, and L comprises a polyether, a polyamine, a polyester, a polyphosphodiester, an alkylene, or a combination thereof.

10. (canceled)

11. (previously presented) The method according to claim 9, wherein L is a polyether and the polyether comprises a polyethylene glycol, a polyalcohol, a propylene glycol, or a combination thereof.

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12. (previously presented) The method according to claim 9, wherein the RNA comprising the structure  $X_1$ -L- $X_2$  comprises an overhang.
13. (previously presented) The method according to claim 12, wherein the overhang comprises 1 to 5 nucleotides.
14. (previously presented) The method according to claim 13, wherein the overhang is a 3' overhang.
15. (previously presented) The method according to claim 13, wherein the overhang is a 5' overhang.
16. (previously presented) The method according to claim 9, wherein the RNA comprising the structure  $X_1$ -L- $X_2$  comprises a left hairpin.
17. (previously presented) The method according to claim 9, wherein the RNA comprising the structure  $X_1$ -L- $X_2$  comprises a right hairpin.
18. (previously presented) The method according to claim 9, wherein the RNA comprising the structure  $X_1$ -L- $X_2$  comprises a bulge.
19. (previously presented) The method according to claim 18, wherein the bulge is a stem loop bulge.
20. (previously presented) The method according to claim 18, wherein the bulge comprises a single uridine residue opposing a double uridine residue.
21. (previously presented) The method according to claim 9, wherein  $X_1$  or  $X_2$  is 100% complementary to the target mRNA.

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22. (previously presented) The method according to claim 9, wherein L is covalently attached to X<sub>1</sub> and to X<sub>2</sub> via an ether, an ester, a carbamate, a phosphate ester, or an amine linkage.

23. (currently amended) A method for inhibiting a target mRNA, comprising:

a) providing an RNA comprising the structure X<sub>1</sub>-L-X<sub>2</sub>, wherein X<sub>1</sub> and X<sub>2</sub> are nucleotide sequences having sufficient complementarity to one another to form a double-stranded stem hybrid and L is a loop region comprising a non-nucleotide linker molecule, wherein at least a portion of one of the nucleotide sequences located within the double-stranded stem is complementary to a sequence of the target mRNA; and

b) contacting the RNA comprising the structure X<sub>1</sub>-L-X<sub>2</sub> with a sample *in vitro* containing or suspected of containing the target mRNA under conditions that favor transfection of the RNA comprising the structure X<sub>1</sub>-L-X<sub>2</sub> into a cell comprising the target mRNA whereby presence of the RNA comprising the structure X<sub>1</sub>-L-X<sub>2</sub> decreases expression of the target mRNA;

wherein X<sub>1</sub> and X<sub>2</sub> each independently comprise between about 19 to 27 nucleotides; L comprises a polyether, a polyamine, a polyester, a polyphosphodiester, an alkylene, or a combination thereof; L is 10-24 atoms in length; the RNA comprising the structure X<sub>1</sub>-L-X<sub>2</sub> comprises a left hairpin RNA; and the left hairpin RNA comprising the structure X<sub>1</sub>-L-X<sub>2</sub> comprises an overhang of 1 to 5 nucleotides and at least one bulge.

Please add the following new claim:

24. (new) A method for assaying whether a gene product is a suitable target for drug discovery comprising:

a) introducing an RNA which targets an mRNA of a gene for degradation into a cell *in vitro*, wherein said RNA comprises the structure X<sub>1</sub>-L-X<sub>2</sub>, wherein X<sub>1</sub> and X<sub>2</sub> are nucleotide sequences having sufficient complementarity to one another to form a double-

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stranded stem hybrid and L is a flexible loop region comprising a non-nucleotide linker molecule of 10-24 atoms in length, wherein at least a portion of one of the nucleotide sequences located within the double-stranded stem hybrid is complementary to a sequence of said mRNA, wherein  $X_1$  and  $X_2$  each independently comprise between about 19 to 27 nucleotides, and L comprises a polyether, a polyamine, a polyester, a polyphosphodiester, an alkylene, or a combination thereof;

b) maintaining the cell of a) under conditions in which degradation of the mRNA occurs, resulting in decreased expression of the gene; and

c) determining the effect of the decreased expression of the gene on the cell, wherein if decreased expression has an effect, then the gene product is a target for drug discovery.